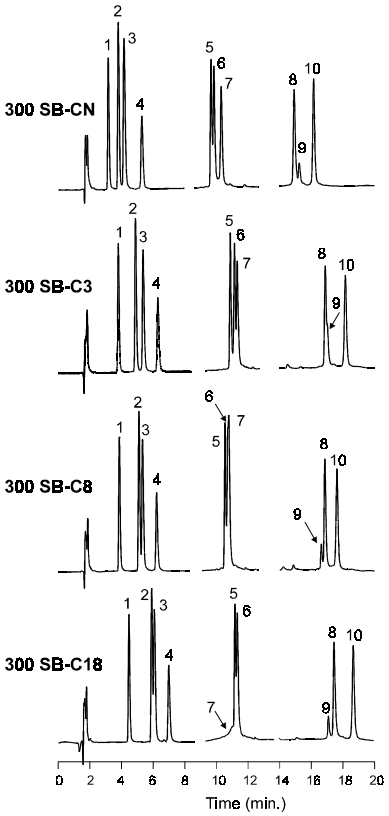
Effect of Bonded-Phase on Resolution and Retention of Polypeptides

Application

Biochemical

Robert Ricker

Reversed-phase HPLC continues to be the analysis technique of choice for the analysis of proteins and peptides. The mobile phase for these separations generally contains aqueous buffers with ACN and TFA. Selectivity changes between samples are often accomplished with the use of different bonded phases.



1. Met Enkephalin
2. Leu Enkephalin
3. Angiotensin II
4. Neurotensin
5. Insulin (BOV)
6. Insulin B chain
7. Cytochrome C
8. Myoglobin
9. Calmodulin
10. Carbonic Anhydrase

Conditions:
ZORBAX 300 SB-CN, SB-C3, SB-C8, SB-C18
(4.6 x 150 mm, Agilent P/N: 883995-905, 883995-909, 883995-906, 883995-902)
Mobile Phase: 15-53% in 20 min.
A: 5:95 ACN:Water with 0.10 % TFA (v/v%) B: 95:5 ACN:Water with 0.085% TFA (v/v%)
Injection 10µL, 2-6µg protein (in 6M Guanidine HCl pH 7.0), 1.0mL/min., 35° C, Detect. UV (215 nm)

Highlights

- *Differences in bonded-phase selectivity can significantly improve resolution in reversed-phase analysis of peptides and proteins. See change in separation between peaks 2 and 3, peaks 5 and 6, and peaks 8 and 9.*
- *Change of temperature, in addition to change of bonded phase, can be used to obtain desired separations.*
- *Sterically-protected bonded phases of ZORBAX SB packings are well suited to chromatography at very low pH.*

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